View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Faculty of Chemistry Repository - Cherry

Arch Biol Sci. 2019;71(1):21-26 https://doi.org/10.2298/ABS180608041K

# Leaf epicuticular waxes of eleven *Euphorbia* species (Euphorbiaceae) from the central Balkans: Impact on chemotaxonomy

Gordana Krstić[1], Ivana Aljančić[2], Jovana Stanković[2], Mirjana Cvetković[2], Petar Marin[3], Peđa Janaćković[3] and Vele Tešević[1,*]

[1] *University of Belgrade – Faculty of Chemistry*, Studentski trg 12-16, 11000 Belgrade, Serbia

[2] *University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Center for Chemistry*, Njegoševa 12, 11001 Belgrade, Serbia

[3] *University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac"*, Studentski trg 16, 11000 Belgrade, Serbia

***Corresponding author**: vtesevic@chem.bg.ac.rs

**Received**: June 8, 2018; **Revised**: August 31, 2018; **Accepted**: September 12, 2018; **Published online**: September 21, 2018

**Abstract:** The presence of *n*-alkanes, free alcohols and free acids in leaf epicuticular wax extracts of 22 samples of 11 *Euphorbia* L. species belonging to the sections *Paralias*, *Esula*, *Myrsiniteae* and *Helioscopia*, 10 of which were never examined before, were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), and *n*-alkane C27 was detected as the principal component of leaf epicuticular waxes in the majority of the examined species, while the most abundant free alcohol was C26. Three *Euphorbia* species belonging to section *Helioscopia* were characterized by a predominance of alcohol C28. Free acid (C16) was the major component in 21 investigated samples. The usefulness of *n*-alkanes and free alcohols and free acids as potential chemotaxonomic markers is briefly discussed.

**Keywords:** *Euphorbia* spp.; leaf epicuticular waxes; *n*-alkanes; free alcohols; free acids

## INTRODUCTION

The genus *Euphorbia*, with more than 1900 species distributed throughout the world, mainly in tropical, subtropical and warm temperate areas [1], is one of the largest and most diverse genera among the Euphorbiaceae family, but also in the whole Embryophyta. *Euphorbia* species are monoecious or dioecious herbs, either geophytic, succulents, shrubs or trees, with a corrosive milky latex. Stems are often spiny, and many species are cactoid. Arrangements of leaves on the stem are spiral, opposite or whorled. Flowers are in cyathia, without perianth members. The male flowers are reduced sometimes to one stamen, surrounding one female (sometimes absent) flower with a gynoecium of three carpels that mature before the male flowers. The whole structure is surrounded by a 'whorl' of green perianth-like bracts with four horn-shaped glands between them, probably representing stipules or bracts. *Euphorbia* plants have explosive fruits (occasionally drupes). Seeds are dispersed by ants, birds, the wind, etc. [1]. Out of 105 *Euphorbia* species found in Europe [1], 73 are documented on the Balkan Peninsula [2].

Leaf epicuticular wax, as an essential barrier, is a very important component of terrestrial plants [3-4]. A characteristic epicuticular wax composition, especially *n*-alkanes, is found among different kinds of plants and has been suggested as a potential character for chemotaxonomy [5-9]. Leaf wax *n*-alkanes have successfully been used as taxonomic characters for some plants, mostly at the genus and species levels [10-14]. Earlier investigations of various vascular plant groups showed that *n*-alkane composition could be useful as an additional chemotaxonomic character at different levels [10-11,15-17]. It was shown that *n*-alkanes from leaf waxes and their average chain length (ACL) could be considered as taxonomic markers for the separation of the subgenera *Dendrocalamus* and *Bambusa* [18]. The dominant *n*-alkanes and their ACL values also served as the criterion for determination of the evolutionary stages of *Dendrocalamus, Dendrocalamopsis* and *Bambusa* species [18].

© 2019 by the Serbian Biological Society

**How to cite this article:** Krstić G, Aljančić I, Stanković J, Cvetković M, Marin P, Janaćković P, Tešević V. Leaf epicuticular waxes of eleven *Euphorbia* species (Euphorbiaceae) from the central Balkans: Impact on chemotaxonomy. Arch Biol Sci. 2019;71(1):21-6.

Waxes extracted from the leaves of five *Euphorbia* species were analyzed for their contents of alkanes, esters, aldehydes, free alcohols and acids [19]. As constituents of epicuticular waxes of several *Euphorbia* species, apart from common lipid wax constituents, triterpenols and ketones [20], branched hydrocarbons [21] and triterpenoids [22] were detected and analyzed. Apart from *E. cyparissias* L. [20-21], none of the other ten studied *Euphorbia* species have previously been examined for their leaf epicuticular wax contents.

Continuing our work on specialized metabolites of *Euphorbia* species [23-24], as well as investigation of leaf epicuticular waxes as a useful source of chemotaxonomic markers such as *n*-alkanes [25-26], the aim of this study was to analyze the *n*-alkanes, free alcohols and free acids of eleven *Euphorbia* species (ten studied for the first time) that grow wild in the central Balkans. The analyzed species belong to four sections of the genus *Euphorbia*: *Paralias*, *Esula*, *Myrsiniteae* and *Helioscopia* [27]. Additionally, in search of new valuable chemotaxonomic characters, the composition of the analyzed metabolites of these species were compared within four *Euphorbia* sections, *Paralias*, *Esula*, *Myrsiniteae* and *Helioscopia*.

## MATERIALS AND METHODS

### Plant material

Twenty-two samples of 11 *Euphorbia* L. species were collected in lowland and mountain regions of Serbia, Montenegro and the FYROM (Macedonia) 2010-2012 (Supplementary Table S1). Voucher specimens were deposited in the Herbarium of the University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac" (accession numbers: BEOU 16858-16879).

### Chemicals and reagents

Organic solvents, dichloromethane ($CH_2Cl_2$), hexane and methanol and the reagent for trimethyl silylation (*N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA)) were purchased from Sigma-Aldrich Co. Column chromatography (CC) was performed on a silica gel 70-230 mesh, ASTM, Merck. Silica gel 60 F254 precoated aluminum sheets (0.25 mm, Merck) for thin layer chromatography (TLC) were used.

### Extraction and fractionation

Samples of fresh whole leaves (1 g) were briefly washed with $CH_2Cl_2$ (20 mL) and the solvent evaporated to dryness to obtain crude extracts (4-14 mg). The complexity of the crude extracts was established by TLC and GC/MS analysis. Fractionation of each crude extract was performed by column chromatography (CC) on silica gel. Elution with hexane (20 mL) afforded a less polar fraction (0.2-11 mg) that was examined directly by GC/MS. Further elution with more polar solvents, $CH_2Cl_2$ (20 mL), followed by 2% methanol in $CH_2Cl_2$ (10 mL), yielded a more polar fraction (1.4-11.9 mg) that was derivatized with BSTFA. Each sample was dissolved in $CH_2Cl_2$, BSTFA was added, and the mixture was kept for 30 min at 70°C. A typical procedure for derivatization is given in the product specification [28]. The product was analyzed by GC/MS. From the extracts of leaf epicuticular waxes, two fractions of different polarities (a *n*-alkane fraction and a fraction consisting of free alcohols and free acids) were isolated and analyzed by GC and GC/MS.

### GC-FID and GC/MS analyses

GC-FID and GC/MS analyses were carried out with an Agilent 7890A apparatus equipped with an autoinjection system (Agilent 7683B Series), an inert 5975C XL EI/CI mass-selective detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology 2-way splitter with make-up, and a *HP-5 MS* fused-silica capillary column (30 m×0.25 mm, film thickness 0.25 μm). The oven temperature was programmed linearly, rising from 60 to 300°C at 3°C/min and then isothermal at 315°C for 10 min; injector temperature was 250°C; detector temperature was 300°C; source temperature was 230°C; quadrupole temperature was 150°C; carrier gas: He; 16.255 psi, constant pressure mode. Samples (1 μL) were injected in splitless mode. Electron-impact mass spectra (EI-MS; 70 eV) were acquired over the *m*/*z* range 30-550. Solvent delay was 3 min. The components were identified based on the comparison of their retention indices (*RI*s) with those of reference spectra (Wiley and NIST databases) as well as by the retention time locking (RTL) method and comparison with the RTL Adams database. The *RI*s were experimentally determined using the standard method [29] related to the $t_R$ of *n*-alkanes injected after the sample under the same

**Table 1.** Relative concentrations of odd *n*-alkanes (as the percentage of total hydrocarbons), calculated CPIs and ACLs in leaf epicuticular waxes of the studied *Euphorbia* species.

| Species | Labels | C23 | C25 | C27 | C29 | C31 | C33 | CPI[a] | ACL[b] |
|---|---|---|---|---|---|---|---|---|---|
| *E. seguieriana* Neck. subsp. *niciciana* | ESN1 | 2.31 | 6.91 | 43.13 | 33.99 | 4.40 | 0.23 | 20.66 | 24.67 |
| | ESN2 | 4.22 | 16.19 | 28.50 | 27.82 | 14.82 | 0.85 | 22.78 | 24.68 |
| *E. seguieriana* Neck. subsp. *seguieriana* | ESS1 | 0.88 | 3.22 | 51.75 | 31.56 | 7.83 | 0.39 | 33.39 | 26.49 |
| | ESS2 | 2.80 | 9.44 | 43.88 | 29.72 | 7.91 | 0.81 | 24.85 | 25.64 |
| *E. nicaeensis* All. | EN1 | 1.42 | 6.90 | 34.99 | 47.89 | – | 1.52 | 15.29 | 25.56 |
| | EN2 | 12.82 | 39.16 | 20.09 | 13.34 | 2.59 | 0.99 | 28.49 | 20.21 |
| | EN3 | 1.81 | 4.22 | 28.79 | 48.56 | 8.62 | 1.21 | 23.49 | 25.96 |
| *E. pannonica* Host | EP1 | 1.88 | 5.09 | 21.22 | 64.99 | 2.46 | 0.16 | 39.36 | 26.66 |
| *E. cyparissias* L. | EC1 | 1.36 | 5.30 | 49.20 | 30.46 | 6.57 | 0.47 | 22.22 | 25.63 |
| | EC2 | 1.89 | 5.49 | 45.16 | 33.38 | 7.15 | 0.60 | 19.90 | 25.66 |
| | EC3 | 1.10 | 3.55 | 50.74 | 29.95 | 8.05 | 0.80 | 22.37 | 26.02 |
| | EC4 | 2.91 | 8.72 | 40.38 | 26.92 | 9.73 | 0.57 | 16.51 | 24.09 |
| *E. amygdaloides* L. | EA1 | 1.30 | 8.01 | 55.87 | 22.73 | 2.66 | 0.02 | 21.04 | 25.51 |
| | EA2 | 1.32 | 7.09 | 39.85 | 34.60 | 9.18 | 0.16 | 15.89 | 25.46 |
| *E. salicifolia* Host | ES1 | 1.98 | 8.09 | 76.46 | 0.33 | 5.36 | 0.43 | 17.85 | 24.57 |
| | ES2 | 2.89 | 23.72 | 40.00 | 22.74 | 3.46 | 0.33 | 23.70 | 24.51 |
| *E. lucida* Wald. et Kit. | EL1 | 2.68 | 8.46 | 31.62 | 38.10 | 8.94 | 1.30 | 19.92 | 24.76 |
| *E. myrsinites* L. | EM1 | 1.02 | 5.73 | 8.29 | 21.35 | 49.10 | 4.54 | 13.31 | 26.58 |
| | EM2 | 1.79 | 10.64 | 13.23 | 23.04 | 34.12 | 4.41 | 13.00 | 24.95 |
| *E. glabriflora* Vis. | EG1 | 0.96 | 2.13 | 8.71 | 23.82 | 51.78 | 2.77 | 12.71 | 26.76 |
| *E. palustris* L. | EPa1 | 1.58 | 6.17 | 33.61 | 45.30 | 6.40 | 0.73 | 26.06 | 25.98 |
| *E. polychroma* A. Kern. | EPo1 | 1.98 | 7.54 | 58.53 | 14.77 | 7.42 | 1.00 | 18.08 | 24.60 |

[a]Carbon preference index, CPI=$[(\Sigma C_{23-33})\text{odd}+(\Sigma C_{25-35})\text{odd}]/[2x(\Sigma C_{24-34})\text{even}]$;
[b]Average chain length, ACL=$\Sigma[c_i]xi)/\Sigma[c_i]$ for i=23-35, with $c_i$ as the abundance of the n-alkane containing *i* carbon atoms.

chromatographic conditions. The relative abundance of the n-alkanes (Table 1) was calculated from the signal intensities of the homologs in the GC-FID traces.

### Calculation of CPI and ACL Values

The carbon preference indices (CPIs) were calculated by using the equation of Bray and Evans [30]. The ACL of C25-C33 alkanes was calculated using the equation of Poynter and Eglington [31].

## RESULTS

### Alkanes

All samples exhibited *n*-alkane patterns with a predominance of odd-numbered homologs in the range C23-C33. The dominant constituents belonging to *n*-alkanes were: C27 (8.29-76.46%), C29 (13.34-64.99%) and C31 (2.46-51.78%) (Table 1), except for one sample (EN2) of *E. nicaeensis*, where the most abundant alkane was C25 (39.16%). Nevertheless, C27 *n*-alkane predominated in five *Euphorbia* species: *E. seguieriana* subsp. *niciciana* and *E. seguieriana* subsp. *seguieriana*, *E. cyparissias*, *E. amygdaloides*, *E. salicifolia*, and *E. polychroma*. In *E. nicaeensis* (EN1 and EN3), *E. pannonica*, *E. lucida* and *E. palustris*, the major *n*-alkane was C29. In the remaining two species, *E. myrsinites* and *E. glabriflora*, the most abundant *n*-alkane was C31.

Regarding the comparison of *n*-alkane composition in analyzed representatives of section *Paralias*, C27 *n*-alkane was predominant in *E. seguieriana*, while in *E. nicaeensis* and *E. pannonica* C29 *n*-alkane was dominant. In section *Esula*, three species (*E. cyparissias, E. amygdaloides* and *E. salicifolia*) were characterized by *n*-alkane C27, and one species (*E. lucida*) by *n*-alkane C29. In *E. myrsinites* from section *Myrsiniteae*, *n*-alkane C31 was the major compound. In section *Helioscopia*, different dominant *n*-alkanes were found in all three

**Table 2.** Relative concentrations of even free primary alcohols and fatty acids (as the percentage of total primary alcohols and fatty acids, respectively) in leaf epicuticular waxes of studied *Euphorbia* species.

| Species (from one or several locations) | Alcohols | | | | Acids | | | |
|---|---|---|---|---|---|---|---|---|
| | C18 | C24 | C26 | C28 | C16 | C18 | C22 | C24 |
| *E. seguieriana* Neck. subsp. *niciciana* | 0.53 | 5.83 | 66.45 | 12.11 | 27.87 | 7.31 | 4.46 | 43.22 |
| | 0.98 | 3.22 | 94.55 | 0.45 | 55.71 | 11.74 | 7.82 | 4.06 |
| *E. seguieriana* Neck. subsp. *seguieriana* | 2.66 | 3.68 | 81.32 | 6.13 | 48.50 | 14.53 | 8.46 | 4.34 |
| | 1.62 | 2.84 | 89.35 | 1.82 | 57.49 | 10.08 | 6.39 | 6.52 |
| *E. nicaeensis* All. | 26.48 | – | 41.10 | 7.13 | 44.78 | 8.43 | 3.13 | 2.37 |
| | 0.26 | 0.52 | 78.21 | 20.91 | 35.26 | 20.63 | 13.19 | 11.35 |
| | 23.50 | | 31.04 | 11.51 | 47.26 | 13.32 | 2.63 | 6.28 |
| *E. pannonica* Host | – | 1.48 | 83.60 | 13.20 | 30.75 | 10.58 | 17.58 | 19.96 |
| *E. cyparissias* L. | 1.77 | 3.45 | 90.10 | 0.92 | 55.71 | 11.74 | 7.82 | 4.06 |
| | 0.99 | 2.05 | 92.59 | 1.08 | 57.80 | 11.44 | 6.92 | 4.11 |
| | 1.79 | 4.00 | 89.66 | 0.59 | 55.34 | 12.18 | 7.15 | 5.03 |
| | 0.80 | 2.45 | 94.41 | 0.49 | 52.32 | 10.51 | 9.12 | 5.27 |
| *E. amygdaloides* L. | 1.49 | 2.68 | 91.05 | 0.28 | 42.36 | 13.35 | 5.79 | 9.04 |
| | 0.83 | 6.29 | 89.18 | 0.33 | 52.07 | 1106 | 10.41 | 5.95 |
| *E. salicifolia* Host | 1.71 | 2.98 | 88.76 | 1.90 | 57.49 | 10.11 | 6.42 | 6.17 |
| | 0.98 | 15.73 | 75.68 | 0.63 | 46.54 | 9.96 | 10.95 | 11.17 |
| *E. lucida* Wald. et Kit. | – | 1.74 | 95.38 | 1.85 | 43.55 | 27.32 | 10.82 | 6.65 |
| *E. myrsinites* L. | 0.56 | 6.66 | 84.66 | 5.56 | 40.86 | 12.15 | 2.88 | 13.56 |
| | 0.58 | – | 92.60 | 4.46 | 48.70 | 9.30 | 5.64 | 3.45 |
| *E. glabriflora* Vis. | – | 13.67 | 13.50 | 65.09 | 43.95 | 11.07 | 8.24 | 3.66 |
| *E. palustris* L. | 1.70 | 1.52 | 20.45 | 69.38 | 57.41 | 6.82 | 11.42 | 3.18 |
| *E. polychroma* A. Kern. | 4.2 | 2.53 | 29.071 | 52.72 | 43.02 | 11.66 | 9.65 | 4.28 |

species: C31 in *E. glabriflora*; C29 in *E. palustris* and C27 in *E. polychroma*. The CPIs were used to express the predominance of odd over even carbon *n*-alkanes, and the ACLs of the C25-C33 *n*-alkanes were calculated, and their values are compiled in Table 1. The values of ACL were in the range 20.21-26.76, while CPI>1 indicated the presence of odd carbon *n*-alkanes.

### Free alcohols and free acids

Free alcohols were predominantly present as even-carbon-numbered alcohols (Table 2). Free alcohols C26 (13.50-95.38%) and C28 (0.28-69.38%) prevailed. In eight of the studied *Euphorbia* species, C26 alcohol was the most abundant, while C28 was the predominant alcohol in three species (*E. glabriflora*, *E. palustris* and *E. polychroma*).Two samples of *E. nicaeensis* possessed lower amounts of C26 alcohol (41.10% and 31.04%, respectively) and were the only examined samples with remarkable amounts of C18 alcohol (26.48 and 23.50%, respectively). *E. nicaeensis* also contained a large amount of C26 alcohol (78.21%) and a very low amount of alcohol C18. The highest amounts of alcohol C26 were detected in *E. lucida* 95.38%, *E. myrsinites*, 84.66 and 92.60%. Even-carbon-numbered free fatty acid was predominant, identified and quantified as a TMSi derivative. The predominant free alcohol of species belonging to section *Helioscopia* was C28 compared to all the other investigated species belonging to different sections (*Paralias*, *Esula* and *Myrsiniteae*), which were characterized by the presence and even predominance of C26 alcohol. In all examined samples of *Euphorbia* species, C16 acid was the most abundant (27.87-57.80%), except in *E. seguieriana* Neck. subsp. *niciciana* where C24 acid was predominant (43.22%).

## DISCUSSION

To date, many different attempts, besides morphological features, have been made to assist in the infrageneric classification of the genus *Euphorbia*. They have included, beside chromosome number [32] and different specialized metabolites (terpenes, alcohols and acids from the latex), which were shown to possess a sys-

tematic significance [33-36]. In the largest systematic survey of the *Euphorbia* genus, triterpenoid profiles for infrageneric classification were used by Anton [37]. Also, Evans and Kinghorn [38] performed a comparative phytochemical investigation of diterpenes of some *Euphorbia* and *Elaeophorbia* species. Webster [39], based on morphology, proposed that *Elaeophorbia* should be reclassified as a subgenus of *Euphorbia*. Finally, *Elaeophorbia* species produce ingenol in the latex, which supports segregation from the genus *Euphorbia* [37]. Also, Acharya and Radhakrishnaiah [40] studied 10 species of *Euphorbia* from a chemotaxonomical point of view. Siegler [41] considered that all phytochemicals (alkaloids, glucosinolates, cyanogenic glycosides, diterpenes, triterpenes, lipids and tannins) are very useful at the generic levels. In their investigation of the epicuticular waxes of five *Euphorbia* species, Hemmers and Gülz [42] concluded that the *n*-alkane profile of *E. nicaeensis* All. could be considered as species-specific. Based on the results of our study, there is no clear distribution of predominant *n*-alkanes within each of the four studied sections. The presented results revealed a rather peculiar distribution of the predominant *n*-alkanes of the investigated species, which does not correspond to current taxonomic (sectional) affiliation.

In general, the ACL values of all investigated species suggest that all species originated at the same time. Also, the high value of certain species means that these species evolved in areas with the highest temperature.

In most of the analyzed *Euphorbia* species (samples from sections Paralias, Esula and Myrsiniteae), free alcohol C26 was the most abundant, while in three species from section Helioscopia the dominant alcohol was C28. Hemmers and Gülz [42], showed that free alcohol C26 prevailed in all investigated *Euphorbia* species, including *E. nicaeensis* and *E. cyparissias*. These results indicate that free alcohols from epicuticular waxes could be considered as potential chemotaxonomic markers. Free fatty acid C16 was dominant in all sections, except in sample 1 of *E. seguieriana* where C14 acid prevailed. This result is in agreement with previous work where also C16 was a dominant fatty acid in the most studied *Euphorbia* species (*E. cyparissias*), contrary to C22 in *E. nicaeensis* [42].

The presented results provide the first insight into the composition of *n*-alkanes, free alcohol and free acid profiles in eleven *Euphorbia* species that grow wild in central Balkans. *Euphorbia* is a large and very complex genus with ambiguous infrageneric taxonomy and a number of species are morphologically (and perhaps chemically) very variable. In spite of intriguing preliminary results, especially based on *n*-alkanes and free alcohols, a relevant chemotaxonomic conclusion requires more extensive sampling and investigations at intra- and interpopulational levels of the species.

**Acknowledgments:** This research was financially supported by the Ministry of Education, Science and Technological development of the Republic of Serbia, (Project 172053).

**Author contributions:** G.K., J.S and M.C. prepared and fractionated the extracts, derivatized the free alcohols and acid fractions, and performed the GC and GC/MS analyses of all samples. V.T. and P.M. collected the plant material, interpreted the experimental data and took part in manuscript preparation. I.A. interpreted the experimental data and took part in manuscript preparation. P.J. interpreted the experimental data and took part in manuscript preparation.

**Conflict of interest disclosure:** There is no actual or potential conflict of interest in relation to this article.

## REFERENCES

1. Mabberley DJ. Mabberley's plant-book: A portable dictionary of plants, their classification and uses. 3rd ed. Cambridge: Cambridge University Press; 2008. 325 p.
2. Turril, WB. The Plant-life of the Balkan Peninsula: A Phytogeographical Study. Oxford : Clarendon Press; 1929.490 p.
3. Baker EA. Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KL, Price CE, editors. The plant cuticle, Linnean Society Symposium Series. London: Academic Press; 1982. 139 p.
4. Jetter R, Schaffer S, Riederer M. Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. Plant Cell Environ. 2000;23(6):619-28.
5. Eglinton G, Hamilton RJ. Leaf epicuticular waxes. Science. 1967;156(3870):1322-34.
6. Jenks MA, Tuttle HA, Eigenbrode SD, Kenneth AF. Leaf epicuticular waxes of the *Eceriferum* mutants in *Arabidopsis*. Plant Physiol. 1995;108(1):369-77.
7. Post-Beittenmiller D. Biochemistry and molecular biology of wax production in plants. Annu Rev Plant Physiol Plant Mol Biol. 1996;47:405-30.
8. Hauke V, Schreiber L. Ontogenetic and seasonal development of wax composition and cuticular transpiration of ivy (*Hedera helix* L.) sun and shade leaves. Planta. 1998;207(1):67-75.
9. Cameron KD, Teece MA, Bevilacqua E, Smart LB Diversity of cuticular wax among *Salix* species and *Populus* species hybrids. Phytochemistry. 2002;60(7):715-25.

10. Vioque J, Pastor J, Vioque E. Leaf alkanes in the genus *Coincya*. Phytochemistry. 1994;36(2):349-52.
11. Maffei, M. Chemotaxonomic significance of leaf wax alkanes in the *Gramineae*. Biochem Syst Ecol. 1996;24(1):53-64.
12. Maffei, M. Chemotaxonomic significance of leaf wax *n*-alkanes in the Umbelliferae, *Cruciferae* and Leguminosae (subf. Papilionoideae). Biochem Syst Ecol. 1996;24(6):531-45.
13. Mimura MRM, Salatino MLF, Salatino A, Baumgratz JFA. Alkanes from foliar epicuticular waxes of *Huberia* species: Taxonomic implications. Biochem Syst Ecol. 1998; 26(5):581-8.
14. Skorupa LA, Salatino MLF, Salatino A. Hydrocarbons of leaf epicuticular waxes of *Pilocarpus* (Rutaceae): taxonomic meaning. Biochem Syst Ecol. 1998;26(5):655-62.
15. Bojović S, Šarac Z, Nikolić B, Tešević V, Todosijević M, Veljić M, Marin PD. Composition of *n*-alkanes in natural populations of *Pinus nigra* from Serbia-chemotaxonomic implications. Chem Biodivers. 2012;9(12):2761-74.
16. Rajčević N, Janaćković P, Dodoš T, Tešević V, Marin PD. Essential-oil variability of *Juniperus deltoides* R. P. Adams along the east Adriatic coast – How many chemotypes are there? Chem Biodivers. 2015;12(1):82-95.
17. Dodoš T, Rajčević N, Tešević V, Matevski V, Janaćković P, Marin PD. Composition of leaf *n*-alkanes in three *Satureja montana* L. subspecies from the Balkan Peninsula: ecological and taxonomic aspects. Chem Biodivers. 2015;12(1):157-69.
18. Li R, Luo G, Meyers PA, Gu Y, Wang H, Xie S. Leaf wax *n*-alkane chemotaxonomy of bamboo from a tropical rain forest in Southwest China. Plant Syst Evol, 2012;298(4):731-8.
19. Hemmers H, Guelz PG. Epicuticular waxes from leaves of five *Euphorbia* species. Phytochemistry. 1986;25(9):2103-7.
20. Guelz PG, Bodden J, Mueller E, Marner FJ. Epicuticular wax of *Euphorbia aphylla* Brouss. ex. Willd., Euphorbiaceae. Z Naturforsch C. 1988;43:19-23.
21. Hemmers WH, Guelz PG, Marner FJ. Tetra- and pentacyclic triterpenoids from epicuticular wax of *Euphorbia cyparissias* L., Euphorbiaceae. Z. Naturforsch C. 1989;44: 563-7.
22. Ahmad W, Nazir M, Chaudhary FM, Ahmad FA. Hydrocarbon distribution in epicuticular waxes of five *Euphorbia* species. Z. Naturforsch C. 1996;51:291-5.
23. Aljančić I, Pešić, M, Milosavljević S, Todorović N, Jadranin M, Milosavljević G, Povrenović D, Banković J, Tanić N, Marković I, Ruždijić S, Vajs V, Tešević V. Isolation and biological evaluation of jatrophane diterpenoids from *Euphorbia dendroides*. J Nat Prod. 2011;74(7):1613-20.
24. Jadranin M, Pešić M, Aljančić I, Milosavljević S, Todorović N, Podolski-Renić A, Banković J, Tanić N, Marković I, Vajs V, Tešević V. Jatrophane diterpenoids from the latex of *Euphorbia dendroides* and their anti-p-glycoprotein activity in human multidrug resistant cancer cell lines. Phytochemistry. 2013;86(2):208-17.
25. Nikolić B, Tešević V, Bojović S, Marin P. Chemotaxonomic implications of the *n*-alkane composition and the nonacosan-10-ol content in *Picea omorika*, *Pinus heldreichii*, and *Pinus peuce*. Chem Biodivers. 2013;10(4):677-85.
26. Nikolić B, Tešević V, Đorđević I, Todosijević M, Jadranin M, Bojović S, Marin P. Variability of *n*-alkanes and nonacosan-10-ol in natural populations of *Picea omorika*. Chem Biodivers. 2013;10(3):473-81.
27. Smith AR, Tutin TG. *Euphorbia* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, editors. Flora Europaea. Cambridge: Cambridge University Press; 1968. p. 213-26.
28. Sigma-Aldrich® [Internet]. Missouri: Sigma-Aldrich Corporation; c 2018. Technical Literature for Derivatization Reagents; [cited 2018 Jun 1]; [about 3 screens]. Available from: Sigma-Aldrich®. St. Louis, Missouri. Sigma-Aldrich Corporation. Technical Literature for Derivatization Reagents [Internet]. Available from: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General_Information/bstfa_tmcs.pdf
29. Van Den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A. 1963;11:463-71.
30. Bray EE, Evans ED. Distribution of *n*-paraffins as a clue to recognition of source beds. Geochim Cosmochim Ac. 1961;22(1):2-15.
31. Poynter J, Eglinton G. Molecular Composition of Three Sediments from Hole 717C: The Bengal Fan. In: Proceedings of the Ocean Drilling Program; 1987 Jul 2–Aug 19; Colombo, Sri Lanka. College Station (Texas): Texas A & M University; c1990. p. 155-61.
32. Perry BA. Chromosome number relationships in the genus Euphorbia. Chron Bot. 1943; 7:413-4.
33. Ponsinet G, Ourisson G. Études chimiotaxonomiques dans la famille des Euphorbiacées-I. Phytochemistry. 1965;4:799-811.
34. Ponsinet G, Ourisson G. Études chimiotaxonomiques dans la famille des Euphobiacées-III. Phytochemistry. 1968;7:89-98.
35. Hegnauer R. Chemotaxonomie der Pflanzen: Eine Übersicht über die Verbreitung und die systematische Bedeutung der Pflanzenstoffe. Basel (Switzerland): Birkhäuser; 1966. Euphorbiaceae; p.103. German
36. Daknley-Gibbs R. Chemotaxonomy of flowering plants. Vol. 3, Orders. Montreal (Canada): McGill-Queens University Press; 1974. 1343-7 p.
37. Anton K. Étude chimiotaxonomique sur le genre *Euphorbia*. [dissertation]. Strasbourg (France): Louis Pasteur University; 1974. 184 p. French
38. Evans FJ, Kinghorn AD. A comparative phytochemical study of the diterpenes of some species of the genera *Euphorbia* and *Elaeophorbia* (Euphorbiaceae). Bot J Linn Soc. 1977;74(1):23-35.
39. Webster GL. The genera of Euphorbiaceae in the southeastern United States. J Arnold Arbor. 1967;48(4):363-430.
40. Acharya H, Radhakrishnaiah M. Some observations on the chemotaxonomy of *Euphorbia*. Acta Bot Ind. 1997;25:219-22.
41. Seigler DS. Phytochemistry and systematics of the Euphorbiaceae. Ann Missouri Bot Gard. 1994;81(2):380-401.
42. Hemmers H, Gülz PG. Epicuticular waxes from leaves of five Euphorbia species. Phytochemistry. 1986;25(9):2103-7.

## Supplementary Data

**Supplementary Table S1**
Available at: http://serbiosoc.org.rs/NewUploads/Uploads/Supplementary%20Table%20S1_3030.pdf